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Report

Nociceptive Sensitization Reduces Predation Risk

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Summary

Sublethal injury triggers long-lasting sensitization of defensive responses in most species examined, suggesting the involvement of powerful evolutionary selection pressures [1]. In humans, this persistent nociceptive sensitization is often accompanied by heightened sensations of pain and anxiety [2]. While experimental [3] and clinical [4] evidence support the adaptive value of immediate nociception during injury, no direct evidence exists for adaptive benefits of long-lasting sensitization after injury. Recently, we showed that minor injury produces long-term sensitization of behavioral and neuronal responses in squid, *Doryteuthis pealei* [5, 6]. Here we tested the adaptive value of this sensitization during encounters between squid and a natural fish predator. Locomotion and other spontaneous behaviors of squid that received distal injury to a single arm (with or without transient anesthesia) showed no measurable impairment 6 hr after the injury. However, black sea bass given access to freely swimming squid oriented toward and pursued injured squid at greater distances than uninjured squid, regardless of previous anesthetic treatment. Once targeted, injured squid began defensive behavioral sequences [7, 8] earlier than uninjured squid. This effect was blocked by brief anesthetic treatment that prevented development of nociceptive sensitization [6, 9]. Importantly, the early anesthetic treatment also reduced the subsequent escape and survival of injured, but not uninjured, squid. Thus, while minor injury increases the risk of predatory attack, it also triggers a sensitized state that promotes enhanced responsiveness to threats, increasing the survival (Darwinian fitness) of injured animals during subsequent predatory encounters.

Results

Because minor injury in the squid, *Doryteuthis (Loligo) pealeii*, induces hypersensitivity to visual and cutaneous stimuli without affecting general activity levels or foraging behavior [5], we hypothesized that nociceptive sensitization in these animals functions primarily to offset increased risks of predation. To test this hypothesis, we staged a series of controlled interactions with a fish predator, black sea bass (*Centropristis striata*), asking whether prevention of nociceptive sensitization by transient anesthetization during surgery influenced the course and outcome of the predatory interaction. Black sea bass are benthic, visual hunters [10] that prey on *D. pealei* as they migrate into shallow, inshore areas [11]. Our observations confirmed that these predatory interactions follow a distinct series of stages that are diagrammed in Figure 1 [5, 7, 8, 12], beginning with a primary defense of crypsis (avoiding detection) and progressing swiftly to secondary defenses against pursuit by the predator that begin with deimatic behavior of rapid body patterning appearance and/or avoidance swimming, followed by escape jetting and protean defense (ink release and erratic, unpredictable jetting directions). When a fish targets a squid, its chase sequence progresses in stages from orientation to pursuit, attack, and capture [8, 13, 14], with many encounters abandoned prior to completion.

Fish Predators Target Squid with Injuries

Minor injury to a single arm 6 hr earlier produced no effects on spontaneous swimming or other behaviors detectable by human observers during video analysis, regardless of whether the animal had been anesthetized during the injury (see also [5]). Nevertheless, black sea bass selectively targeted squid in both injured groups (injured [I] and injured treated with anesthetic [IA]) compared to the uninjured groups (uninjured [U] and uninjured treated with anesthetic [UA]) (for anesthetic details see legend of Figure 2 and Supplemental Experimental Procedures available online). In mixed-treatment trials (n = 4 trials, containing 2 each injured and uninjured squid), squid in the I group were more likely to be captured than squid in the U group present at the same time (five of eight I versus one of eight U squid, odds ratio = 11.7, z = 1.89, p = 0.05). In trials involving single-treatment groups of squid (e.g., all four were uninjured or all four were injured), fish both oriented to and pursued squid in the I and IA groups from longer distances compared to squid in the U and UA groups (post hoc t tests, p = 0.02 and 0.001, respectively; Figures 2A and 2B), indicating that even minor injuries make squid either more conspicuous or more attractive targets to a natural predator [15].

Sensitized Squid Are More Responsive to Predation Threats

Squid in the I group (n = 20) had longer alert distances [16, 17] than squid in the IA, U, or UA groups (n = 16, 20, and 16, respectively), indicating earlier initiation of secondary defense (I versus U, p = 0.03; Figure 2C). Similarly, I group squid showed alert behaviors at earlier stages of encounters than IA, U, or UA squid (p = 0.004; Figure 2D), despite fish orientation and pursuit usually occurring at greater distances from injured than uninjured squid (Figures 2A and 2B). I group squid also had longer flight initiation distances (versus U, p = 0.008; Figure 2E) compared with squid in the other three groups.

Nociceptive Sensitization in Prey Affects the Likelihood that a Predatory Encounter Will Escalate

Squid in the I group were less likely to be pursued after orientation by fish than U group squid (p = 0.046; Figure 2F), compared with squid in the IA group (p = 0.017). In encounters that escalated to pursuit, squid in both the I and IA groups had higher probabilities of being attacked (I versus U, p = 0.042; IA versus U, p = 0.023; Figure 2G). However, only attacks

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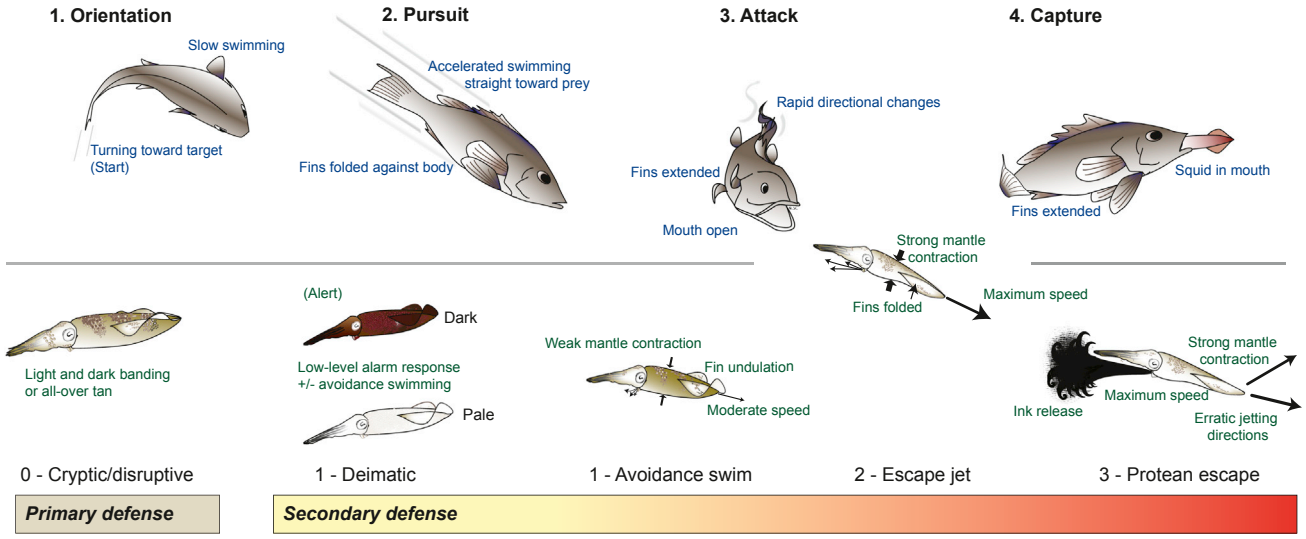

Figure 1. Escalation Pattern in Predator-Prey Interactions between Black Sea Bass and Squid

Top: four stages of predator behavior. Orientation is the first change in direction toward a squid from an ongoing swimming trajectory, and the distance from fish to squid is the "start distance" of the predation attempt. Pursuit is an accelerated, direct approach toward a squid, with the fish's dorsal, pectoral, and caudal fins folded. Attack is close-proximity "grappling," with the fish's mouth open and fins extended to facilitate rapid directional changes. Capture is defined as any part of the squid's body caught in the mouth of the fish. Bottom: defensive responses of squid to the fish. Primary defense (avoiding detection via crypsis) escalates to secondary defenses once the squid is alerted. Crypsis, via chromatophore patterns of disruptive banding while sitting on the substrate or all-over beige when swimming, occurs in the absence of encounters and often during early encounter stages; it received an escalation score of 0. Distance between squid and fish at the first secondary defensive behavior is the "alert distance." Secondary defenses were scored based on their typical progression. Deimatic chromatophore displays that distract or startle a predator were scored 1, as were slow avoidance swimming evoked by distant threat. Escape jetting without inking was scored 2. This typically (but not always) followed expression of behaviors scored 1. Ink release, which was almost always combined with erratic escape jetting, was scored 3. The highest escalation score was recorded for each predatory encounter.

involving IA squid had a significantly greater probability of capture ($p = 0.048$; Figure 2H). Similarly, IA squid made fewer successful escapes (indicated by the total number of encounters that fish aborted prior to capture) than U squid (data not shown, $p = 0.028$).

Predation Risk Is Highest among Squid that Are Injured but Not Sensitized

Prior injury reduced survival of attacked squid, with both the I and IA groups having lower odds of surviving the 30 min encounter compared with squid in the U group (I versus U; odds ratio = 4.89 (large effect), $z = 2.212$, $p = 0.026$, IA; odds ratio = 17.33 (large effect), $z = 3.35$, $p = 0.008$; Figure 3). Based on our earlier findings that injury induces behavioral sensitization [5] and that neuronal sensitization in squid is prevented by injection of anesthetic at an injury site [6], we predicted that survival in the IA group would be less than that in the I group (i.e., that anesthetic treatment would prevent nociceptive sensitization and its later survival benefit). This prediction was supported by the higher survival rate of I group squid compared to the nonsensitized IA group squid (odds ratio 3.54 [medium effect], $z = 1.618$, $p = 0.05$). This apparent benefit of nociceptive sensitization (Figure 3) is not accounted for by deficits caused by the anesthetic treatment, because there was only a slight, statistically insignificant difference in survival between the UA and U groups.

Discussion

This study provides the first experimental support for the hypothesis that nociceptive sensitization is an adaptive response to injury. Although neural mechanisms of nociceptive sensitization have received extensive study in the contexts of pain [18, 19] and aversive learning [20, 21], and its adaptive value for recuperative behavior, contextual learning, and defense against potential attackers has been conjectured [1, 22–24], until now there has been no direct evidence for a contribution of nociceptive sensitization to Darwinian fitness. Indeed, in clinical contexts long-lasting sensitization and pain are often assumed to be maladaptive rather than adaptive [19, 25].

Injury and other types of noxious stimulation produce several forms of long-lasting behavioral and neural sensitization in many animals [5, 9, 26–29]. At the behavioral level, this includes general sensitization (hypervigilance, anxiety-like states) and site-specific sensitization near sites of injury (expressed in mammals as primary hyperalgesia) [1]. Behavioral sensitization is known to occur after predatory attacks (including conspecific and parasitoid assaults) [3, 6, 30–32]. Even in some invertebrate animals, noxious stimulation can produce long-lasting motivational effects with possible functional similarities to mammalian pain and fear states [33–35]. The existence of nociceptive sensitization and related motivational states in diverse species suggests that long-lasting, injury-related defensive states—which in humans are associated with pain and anxiety—are the product of strong and widespread evolutionary selection pressures [1].

Sublethal injury in animals is ubiquitous and costly [13, 36, 37], arising from failed predatory attacks [38], intraspecific conflicts [39], and injury unrelated to predatory attempts [40]. A significant fitness cost of injury is increased risk of subsequent attacks by predators [41, 42], which often target compromised prey [15, 43, 44]. Injury may also lead to less successful avoidance of predation because of biomechanical [45] or energetic sequelae of injury [39, 46].

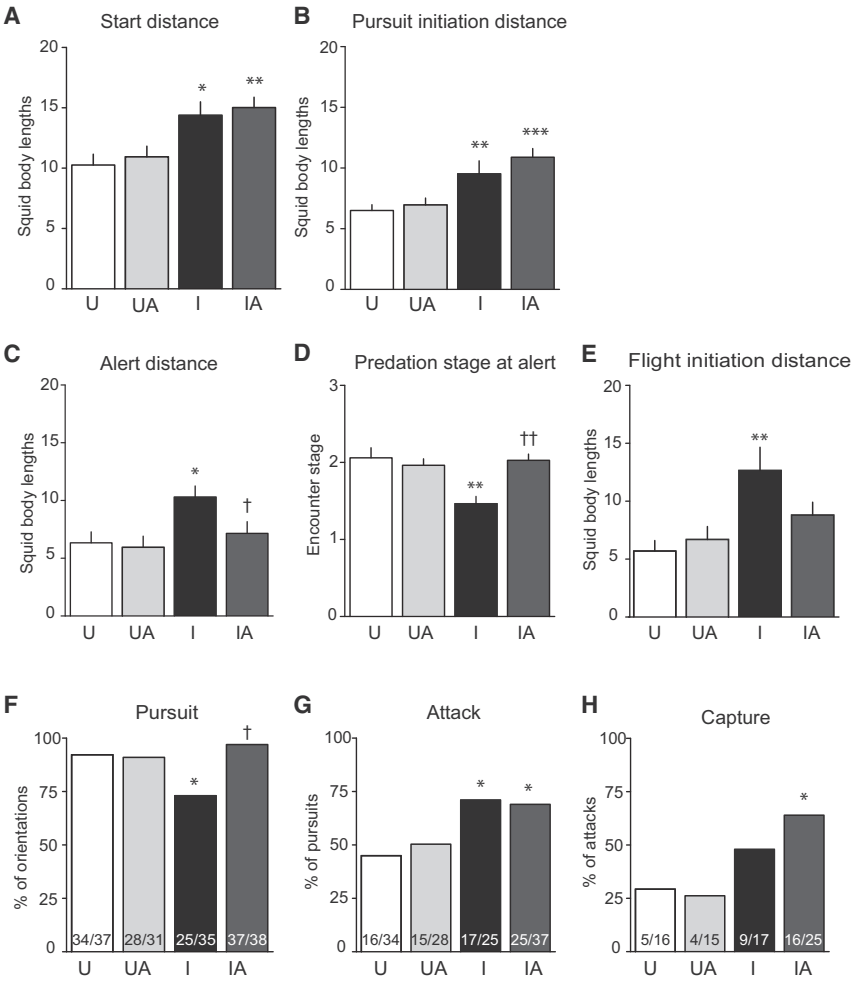

Figure 2. Encounters Are Affected by Previous Injury to Prey and by Nociceptive Sensitization

Squid received one of four treatments 6 hr prior to exposure to fish. Uninjured (U, n = 20) squid were handled but not injured. Uninjured under anesthetic (UA, n = 16) squid received general and local anesthesia (immersion in 1% ethanol and local injection of isotonic $MgCl_2$ into an arm) but no arm injury. Injured (I, n = 20) squid were handled and the tip of one arm was removed with a surgical scalpel. Squid injured under anesthetic (IA, n = 16) received general and local anesthesia before arm injury.

(A and B) Fish targeted injured prey. Orientation to squid (start distance; A) and initiation of pursuit (B) occurred at greater distances in both the I and IA groups compared with U group. The UA group was not different from the U group. Bars show mean + SEM. Two-way ANOVA with post hoc, two-tailed t tests. *, comparisons to U group; +, comparisons between the I and IA group. *p < 0.05, **p < 0.01, ***p < 0.001.

(C, D, and E) Squid in the I group had longer alert distances (earlier initiation of secondary defenses; C), were alerted at earlier encounter stages (D), and initiated flight (escape jetting with or without ink) at greater distances compared to the other groups (E) (Kruskall-Wallis test with post hoc, Bonferroni-corrected Mann-Whitney U tests, significance indicators as for B).

(F, G, and H) The probability of encounter escalation was increased by prior injury and by prior anesthetic treatment. Encounters were less likely to escalate from orientation to pursuit for the I group than for the other groups (F), were more likely to escalate to attack for the I and IA groups (G), and were more likely to escalate to capture for the IA group (H). Fisher's exact tests, significance markers as for (D). Ratios indicate number of escalations/number of encounters.

The adaptive value of nociceptive sensitization was revealed in our study by using brief anesthetic treatment at the time of injury—which prevents the development of nociceptor sensitization in *D. pealeii* ([6] see also [9])—to prevent ensuing hypervigilance. Adaptive value was indicated by reduced responsiveness of these squid to the predator compared with squid injured without anesthetic (Figures 2C–2E). Most importantly, the previously anesthetized, injured squid had the highest likelihood of capture (Figure 2H) and mortality during the predatory interaction (Figure 3), revealing that the full cost of injury is partially compensated by sensitized behavior. Several considerations indicate that these effects result from anesthesia preventing the development of generalized nociceptive sensitization [5, 6], rather than nonspecific, lingering effects on the condition of the animal. Anesthetic treatment by itself did not compromise behavioral function because the UA group showed no significant differences from the U group in any of the behavioral measures (Figures 2C–2E) or survival (Figure 3), nor did previously anesthetized animals show other signs of motor or sensory impairment during the predatory encounter. In addition, very brief, light general anesthesia by immersion in 1% ethanol [47] was combined with injection of isotonic $MgCl_2$ locally at the site to be transected, where the restriction of relaxed chromatophores to skin near the injection site showed that the $MgCl_2$ did not travel to the base of the treated arm. Moreover, we have shown previously that $MgCl_2$ injected near a wound on a fin remains localized to the injury site while completely blocking the development of generalized sensitization of primary nociceptors [6]. Therefore, the prevention of hypervigilance and the increase in mortality can be attributed to our anesthetic procedures selectively blocking the induction of long-lasting nociceptive sensitization during arm injury. An interesting possibility is that the adaptive, injury-induced hypervigilance may be promoted by persistent spontaneous activity generated in peripheral terminals of primary nociceptors of the squid [6].

Higher mortality rates among injured animals might also be explained by debilitating physiological consequences of injury ([46, 48] but see [49]). However, consistent with our earlier results [5], we found no evidence that injured squid had shorter flight distances or flight durations after encounters were aborted by fish, which would be expected if the experimental injury were debilitating. Loss of maneuverability due to arm injury is also unlikely, as only the injured squid lacking sensitization were less effective at averting predatory escalation from attack to capture, the stage when rapid changes in direction would be most critical.

Although we observed no deficits in swimming ability, squid with injuries, whether or not they were sensitized, had lower survival rates compared with uninjured squid. This confirms that even minor injuries carry significant costs [50], but it is not yet clear which consequences of the arm injury were

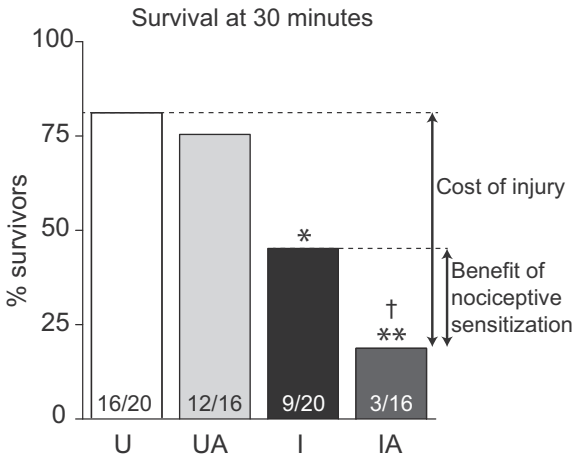

Figure 3. Injured Squid Lacking Nociceptive Sensitization Had the Lowest Odds of Survival

At the conclusion of a 30 min trial with free interaction of squid and fish, squid in the I and IA groups had lower overall survival than in the U group, and IA group squid were most likely to be killed. The difference in survival between the U and the IA group can be considered the cost of being injured, while the difference in survival percentage between the IA and I groups ($p = 0.05$) reveals the benefit that nociceptive sensitization provides to injured animals. Odds ratios, *$p \leq 0.05$, **$p < 0.01$. U, uninjured; UA, uninjured with anesthetic treatment; I, injured; IA, injured with anesthetic treatment.

responsible for this increased risk. The differing survival odds among squid with and without injuries or sensitization resulted from changes in behavior of both the predator and prey. In squid, sensitization produced changes in antipredator responses that resulted in increased alert distances and flight initiation distances and increased reliance on escalated defensive behaviors such as inking and jetting at earlier stages of encounters (Figure 1) [8]. Theoretical and empirical studies have shown that flight initiation distance should be longer when prey perceive risks to be greater [16], which is consistent with our observations. However, these same studies also predict that flight initiation distance should be longer when initial fitness (survival probability at the start of an encounter) is higher. This prediction differs from our finding that injured squid had longer flight initiation distances despite the demonstrated fitness cost of injury. Therefore, in some species, early flight and related effects of nociceptive sensitization may occur instead of or in opposition to other responses of prey animals to substandard conditions such as previous injury.

Fish predators also behaved differently in encounters with injured squid, regardless of prey sensitization. Fish targeting injured prey had longer start distances and initiated pursuits from greater distances compared with controls, suggesting that they may invest more effort in encounters with prey perceived to be more vulnerable. Encounters with injured squid were also more likely to escalate to attack compared with encounters with control squid. Previous studies have shown that predators are adept at detecting and targeting prey animals in substandard condition [15, 44, 51]; an interesting question is whether fish targeted injured squid because they detected signs of injury directly via visual or olfactory cues, or whether injured squid made themselves more conspicuous at early encounter stages by abandoning crypsis sooner [48]. Although video analysis of the injured squid did not reveal deficits in swimming speed or maneuverability, it is likely that predators that have evolved to select the most vulnerable prey are capable of identifying weaknesses that are not apparent to human observers.

The necessarily artificial conditions of our experimental encounters, particularly the relatively small size of our experimental arena, may have produced dynamics that are uncommon in the wild. Nonetheless, these experimental conditions of high, unremitting predation risk, combined with an intervention that prevented the development of injury-induced hypervigilance, were sufficient to demonstrate opposing effects of injury and nociceptive sensitization and thereby to reveal an adaptive function for the sensitization. Moreover, our findings strongly support the possibility that mitigation of heightened predation risk after sublethal injury has been an important selection pressure shaping the evolution of persistent neural hyperexcitability mechanisms underlying injury-related states of behavioral hypersensitivity.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.03.043.

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